REMARKS/ARGUMENTS

The elected method claims 10-14 have been carefully amended to move particularly recite the novelty of the present invention, and withdrawn apparatus claims 6-9 remain in the application prior to the filing of a divisional application thereon.

Allowance of the amended claims is courteously solicited for the following reasons.

According to Applicant's invention, an improved cell grown method is provided having multiple applications and includes the basic steps of introducing cells into a closed enclosure, delivering ventilation gases, nutrient media and growth factors to said enclosure, and harvesting the cultivated cells. The invention differs from the prior art in that the method further comprises the steps of providing multiple sources of different nutrient media and multiple sources of different growth factors with connection of said multiple sources to the enclosure by flow rate adjustment means, and selecting compositions and flow rates of mixtures of the different nutrient media and selecting compositions and flow rates of mixtures of the different growth factors to be delivered to the enclosure for an envisaged application, said compositions and flow rates being modifiable at regular or irregular intervals during the process of said application, which modifications can be effected independently of each other.

Applicant courteously contends that it would not be "obvious" (35 U.S.C. § 103) to combine the teachings of the cited reference to anticipate the invention recited in the amended claims. No combination of the references discloses Applicant's inventive concept of combining multiple different sources of nutrient media and multiple different sources of growth factors with independent control means for varying and selecting the compositions and flow rates of the mixture for treating the cells.

The Fei, et al., patent No. 5,635,387 relates to a method for culturing human haematopoietic cells and their precursors, the method consisting in inoculating the

precursor cells into a culture vessel containing a nutritive medium, growth factors and optionally containing plasma or serum.

In the embodiment shown in Fig. 1, a culture vessel 148 and cell separation means 100 are integrated into a single device, in which a column assembly 144 of the cell separator is modified so that the target cells, after release from the column 144, flow into the cell culture vessel 148 by means of tubing 152 connected to an inlet of the culture vessel 148, the culture vessel 148 further comprising an outlet connected to a collection bag 162 by means of another one tubing 152.

The culture vessel is provided with two ports 141, 143, a first port 141 being provided for the purpose of sampling and a second port 143 forming perfusion means by which medium can be added (col. 14, l. 43-46).

This port 143 which can be used for adding a medium into the culture vessel 148 is not connected to a plurality of sources of different nutrient media and of growth factors, the culture vessel 148 is not provided with flow rate adjustment means, etc.

This reference therefore fails to disclose applicant's provision of multiple sources of different nutrient media and different growth factors, together with means for selecting the compositions and the flow rates of mixtures of the nutrient media and mixtures of the growth factors, thereby to produce an improved cell growth.

The Hopkins patent No. 4,468,455 discloses a cell culture process, in which addition of an additive is only carried out after it has been determined that the previous addition of additive has caused at least a minimum change in metabolic activity of the cells.

In the embodiment shown in Fig. 1, the apparatus for growing a cell culture comprises a fermenter vessel 1 which contains the liquid culture medium comprising the cells and a nutrient medium that is subjected to growth conditions, and a metabolic activity sensor 2 connected to a controller 5 capable of generating a control signal of a

pump 6 for adding a given quantity of additive in the fermenter vessel 1. A source of oxygen-containing gas is also connected to the fermenter vessel 1 by means of a valve 12 for regulating gas flow rate.

However, in this reference, a plurality of sources of different nutrient media and growth factors are not connected to the fermenter vessel, and no means are provided for selecting and changing the composition and the flow rate of the nutrient medium and of the growth factors fed to the fermenter vessel. Consequently, the Hopkins patent does not anticipate these essential features of the present invention.

The Vajta patent No. 6,399,375 discloses a method for culturing cells and tissues in closed containers comprising a culture medium and a gaseous atmosphere, the containers being submerged or immersed in thermostatically controlled liquid bath.

In the embodiment shown in Fig. 6, each container is formed with a bag 110 in which the culture medium and the cells to be cultured are introduced therein before the heat sealing of the bag to be ready for submersing or immersing it into the liquid of a liquid incubator, the culture medium comprising nutrients, growth factors and other components in well defined proportions and levels.

In the embodiment shown in Fig. 7, the container further comprises an inlet 116 for introducing a culture gas or gas mixture into the bag after its sealing, and an outlet 118 for releasing excess gas.

However, the container is not provided with further inlet for adding additional or different nutrient media and growth factors therein. Multiple sources of different nutrient media and growth factors cannot be connected to the container, and no means are provided for selecting and changing the composition and the flow rate of the nutrient medium and of the growth factors contained in the container.

The Emerson, et al., patent No. 5,646,043 discloses a method for the ex-vivo replication of human stem cells and/or expansion of human progenitor cells, the method

consisting in rapidly replacing, either continuously or periodically, the liquid culture medium which contains human cells and a nutrient medium, by means of perfusion.

The replacement rate depends on cell density and in a preferred embodiment is effected with the optional addition of supplementary haematopoietic growth factors.

However, only one source of nutrient medium and one source of growth factors are connected to the bioreactor containing the liquid culture medium. Multiple sources of different nutrient media and of growth factors are not provided, and no means are provided for varying the composition and the flow rate of the nutrient medium and of the growth factors during the process of the culture. Consequently, this patent fails to anticipate these essential features of the invention.

The Takeuchi, et al., patent No. 5,304,483, discloses a process for controlling cultivation conditions for animal cells, by measuring the glucose concentration and lactic acid concentration in a culture medium of animal cells and, when the ratio of the formed lactic acid to the consumed glucose is outside a fixed range, controlling the dissolved oxygen concentration and/or glucose concentration in the culture medium.

In the embodiment shown in Fig. 6, a fermenter 1 which contains a culture medium 2 comprising the animal cells, is supplied with oxygen-containing gas by means of a conduit 13 and a flow rate control valve 6, provided thereon. A glucose containing solution is fed through a conduit 14 and a flow rate valve 7. Sensors 8 and 9 measure respectively the glucose and lactic acid concentrations and are connected to an operation control 12 where control signals of the valves 6, 7 are generated.

However, a plurality of sources of different nutrient media and growth factors are not connected to the fermenter, and thus no means are provided for selecting and changing the composition and the flow rate of the nutrient medium and of the growth factors. This reference fails to teach the essential features of the present invention.

The Kearney patent No. 5,424,209 relates to an automated cell culture and testing system which provides a completely self contained environment in which living tissues may be placed and may be nutrified and oxygenated.

As shown in Fig. 8, the system comprises a plurality of bioreactors 8, each bioreactor containing living tissues and comprising an inlet port 96 for selectively communicating with a media reservoir 2 containing nutrient media or a test material reservoir 31 containing drug, hormone or chemical, each individual bioreactor 8 being individually supplied without interconnection with other bioreactors 8 (col. 10, l. 35-52). The bioreactors 8 are also provided with an end port 98 and a side port 99 whereby media flow may exit.

However each bioreactor communicates with only one source of nutrient media and no means are provided for varying the compositions of mixtures of different nutrient media or the mixtures of different growth factors.

Consequently, each bioreactor is connected to one source of nutrient medium. Multiple sources of different nutrient media and of growth factors are not provided, no flow rate adjustment means are provided, and no means for changing the composition and the flow rate of the nutrient media are provided. Thus, the Kearney patent fails to anticipate the invention.

The Palsson, et al., patent No. 5,888,807 relates to a device for monitoring and growing human stem and/or hematopoietic cells, in which diverse cell types are simultaneously-cultured in the presence of appropriate levels of nutrients and growth factors continuously maintained in the device while removing undesirable metabolic products.

As shown in Fig. 2, the device comprises a first bioreactor chamber 70 connected to a media reservoir 54 by a pump 62 and a conduit 68 provided with a media component injection means 82, the media components being test components, additional factors, or

the like. The second chamber 74 of the bioreactor is connected to a sensor 78 by means of a conduit 76, detecting the change in composition of the media. By monitoring the effluent from the chamber, the media introduced into the bioreactor may be modified, the oxygen partial pressure may be changed, the gas flow rate may be altered, various components may be augmented or the global rate of perfusion may be slowed or increased. The media exiting from the bioreactor is returned to the media reservoir 54 (col. 15, l. 55-67 and col. 16, l. 1-20).

This reference teaches therefore that a media can circulate through a bioreactor from a reservoir and return to the reservoir from the bioreactor, means being provided for changing the circulation rate and for adding more or less test components, additional factors or the like to the circulating media. However, this patent does not teach that a plurality of sources of different nutrient media and of different growth factors may be connected to a culture vessel that is supplied with a mixture of the different nutrient media and of the different growth factors. Applicant therefore respectfully contends that the Palsson, et al., patent does not anticipate this essential feature of the invention.

In the Grandics, et al., patent 5,571,720, an integrated cell culture protein purification system is disclosed for the automated production and purification of proteins, comprising a bioreactor subunit having a hallow fiber bioreactor to culture and maintain cells which secrets the desired product into the cell culture medium, which is circulated through a purification cartridge which absorbs the desired products.

In the embodiment shown in Fig. 1, feeding of cells is accomplished by continuous perfusion by introducing fresh and withdrawing spent medium from the bioreactor by means of a pump A.

However, the disclosed system is a purification system and not a culture system. Several sources of different nutrient media and growth factors are thus not connected to

the bioreactor and no means are provided for varying and selecting the composition and the flow rate of nutrient media and of growth factors.

The Pykett, et al., patent No. 6,440,734 discloses a method for the long-term culture of haematopoietic cells, which are cultured on a three-dimensional porous biomaterial such as a porous matrix.

In the embodiment shown in Fig. 1, the cells culture occurs in an apparatus which includes a first cell culture chamber 10 and a second cell culture chamber 12 connected together by a conduit 16 and each containing a porous solid matrix 18. The first chamber 10 communicates with a media input conduit 26 via a port 24 which can be provided with a valve, and with a sample conduit 32 for adding or removing materials from the fist chamber 10 via a sample port 30 which can be provided with a valve. The first chamber 10 also presents an outlet port 34 communicating with the connection conduit 16. The second chamber 12 communicates with a sample conduit 32 and an outlet conduit 36 whereby media circulates, and includes an augmentation conduit 38 far supplying the second chamber 12 with materials, preferably haematopoietic growth factors. These conduits 26, 38 which can be used for adding media and growth factors into the two culture chambers 10, 12 are not connected to a plurality of sources of different nutrient media and of growth factors.

This patent fails to anticipate the present invention wherein the use of multiple sources of different nutrient media is one of the essential patentable features.

In conclusion, no combination of the cited references would cause Applicant's invention to be "obvious" to one skilled inn the art. Since no patent discloses or suggests growing cells in a culture chamber supplied with different nutrient media from multiple sources and different growth factors from multiple sources, in combination with independent separate rate adjustment means, the invention cannot be held to be obvious under 35 U.S.C. § 103.

Applicant's combination of technical features allows one to use the same method and the same device in a plurality of different applications, such as maintenance, proliferation, amplification and differentiation of cells. The invention also permits to modify and select the compositions and the flow rates of nutrient media, the compositions and the flow rates of growth factors, and in a preferred embodiment of the invention also the compositions and the flow rates of ventilation gases, during the cell growing, e.g., in order to increase the culture yield.

The combination as recited in amended parent claim 10 is not obvious for one skilled in the art with respect to all the cited prior documents which disclose only methods and devices adapted and designed for a particular single application. To the contrary, the invention concerns a method with multiple applications, such as maintenance, proliferation, amplification or differentiation of cells, this method also permitting important changes in the cell culture conditions during a specific application, which allow to obtain results which could not be obtained by using the methods and devices disclosed in the cited prior documents.

Accordingly, Applicant courteously believes that the present invention is clearly patentably distinguishable from the cited prior art, and that allowance of the application is warranted.

Favorable action is courteously solicited.

Respectfully submitted,

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